Remarks

The Office Action mailed June 16, 2008, has been received and carefully reviewed. Claims 1 and 21 having been amended, and claims 36-38 and 42 having been canceled herewith, without prejudice, and claim 43 having been added, the pending claims are claims 1-8, 21-29, 32-35, and 43. Reconsideration and withdrawal of the rejections are respectfully requested.

Support for newly added claim 43 is found in the specification at, for example, page 11, lines 5-7 and 14-17.

Claim Objection

The Examiner has objected to claim 21 under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim.

This objection is respectfully traversed. However, in order to advance prosecution, claim 21 has been amended to recite a peptide "conjugated to first and second bioactive compounds, wherein the first bioactive compound comprises biotin" thereby clarifying that the peptide is conjugated to at least two bioactive compounds, one of which is the biotin which accounts for the biotinylation recited in claim 1.

Applicants respectfully request reconsideration and withdrawal of the objection to claim 21 under 37 CFR 1.75(c), as being of improper dependent form.

Rejection under 35 U.S.C. §102(b)

The Examiner has rejected claims 1-8, 21-26 and 32 under 35 U.S.C. 102(b) as being anticipated by Low et al. (WO 90/12096). This rejection is respectfully traversed.

The Examiner asserts that Low et al. teach transmembrane transport of exogenous molecules including proteins and polynucleotides in plant, mammalian, and bacterial/prokaryotic cells. The Examiner further asserts the Low et al. exemplify (a) that biotin conjugated to various proteins was taken up by mammalian and plant cells in the absence of a membrane permeabilizing agent; and (b) that biotin conjugated to plasmid DNA (pUC8) was added to *E.*

coli that was made competent with the addition of $MgCl_2$ and $CaCl_2$, but in the absence of a membrane permeabilizing agent.

Applicants respectfully submit that the Examiner has misread Low et al. with respect to the presence or absence of membrane permeabilizing agents. Both $MgCl_2$ (magnesium chloride) and $CaCl_2$ (calcium chloride) taught in Low et al. are, in fact, membrane permeabilizing agents, as is "Locke's solution" which contains $CaCl_2$ and was used in the mammalian cell experiments reported in Low et al. at page 21, line 21 (cells were "washed with a 20 ml portion of fresh Locke's solution"). See Ferrell and Koshbaten (Antimicrobial Agents and Chemotherapy, 389(5):973-980, 1994), where the contents of a standard Locke's solution are defined on page 571, last full paragraph, and include calcium chloride. A copy of Ferrel and Koshbaten is included with the Information Disclosure Statement submitted herewith.

With respect to $CaCl_2$ (calcium chloride), Applicant's specification teaches at, for example, page 14, lines 19-20, that one can "make cells 'competent' for transfer by pretreating them *with a permeabilizing agent such as calcium chloride*" (emphasis added). Additionally, the chemical datasheet on calcium chloride, as published by CQ Concepts and submitted in the Information Disclosure Statement filed herewith, clearly states that "[a]queous calcium chloride is used in genetic transformation of cells by increasing the cell membrane permeability."

Additionally, Example 12 in Low et al. describes the introduction of biotinylated DNA into *E. coli* (in the presence of membrane permeabilizing agents, it should be noted), Low et al. do *not* have any working examples describing the introduction of a *peptide* into *E. coli* (or any other Gram negative bacterium). In this regard, the Examiner is requested to note that claims 1, 7 and 21 have been amended to delete recitation of a "peptidomimetic." Thus, claim 1 and claims dependent therefrom are not anticipated by Low et al.

Independent claim 3 is drawn to a method for introducing a compound into a Gram negative bacterial cell wherein the cell is contacted with a biotinylated compound in the absence of a membrane-permeabilizing agent. As noted above, Low et al. do not teach introduction of a biotinylated compound into a Gram negative bacterial cell bacterium *in the absence of a*

permeablizing agent. Thus, claim 3 and claims dependent therefrom are not anticipated by Low et al.

Independent claim 5 is drawn to a method for identifying a compound having antimicrobial activity, wherein the method involves contacting a Gram negative bacterial cell with a biotinylated compound, and determining whether it has an antimicrobial effect on the cell. Compounds having antimicrobial effect are described in the specification at, for example, page 12, lines 8-13:

> Antimicrobial compounds are compounds that *adversely affect* a microbe such as a bacterium, virus, protozoan, or the like. Antimicrobial compounds include, for example, inhibitory compounds that slow the growth of a microbe, microbiocidal compounds that are effective to kill a microbe (e.g., bacteriocidal and virocidal drugs, sterilants, and disinfectants), and compounds effective to interfere with microbial reproduction, host toxicity, or the like (emphasis added).

The Examiner asserts that Low et al. determine the antimicrobial effect on the cell by the addition of ampicillin to the transformed cell culture and that colonies that survived the ampicillin treatment were counted. Applicants respectfully submit that the compound that Low et al. introduced into *E. coli* was *not* one that has an antimicrobial effect (i.e., a compound that adversely affects a microbe) on the cell, as recited in claim 5. Rather, Low et al. conferred antibiotic *resistance* on the transformed cell. This is evidenced in the title of Example 12 of Low et al. ("*E. coli* transformation and expression of ampicillin resistance gene") as well as in the methodology of counting colonies that *survive* the ampicillin treatment. Applicants submit that antibiotic resistance would *advantageously* affect a microbe rather than adversely affect a microbe. Low et al. did not determine the antimicrobial effect on the cell; instead, they determined the antibiotic resistance of the transformed cell. As such, Low et al. fail to teach each and every element of independent claim 5. Thus, Applicants respectfully submit that claim 5, and claims depending therefrom, are not anticipated by Low et al.

For at least the reasons given above, Applicants submit that Low et al. fail to teach each and every element of the rejected claims. Applicants respectfully request reconsideration and

withdrawal of the rejection of claims 1-8, 21-26 and 32 under 35 U.S.C. 102(b) as being anticipated by Low et al.

The Examiner has rejected claims 1-8, 22-26, 29 and 32-35 as being clearly anticipated by Dargis et al. (Antimicrobial Agents and Chemotherapy, 389(5):973-980, 1994). The Examiner asserts that Dargis et al. teach biotin linked beta lactam antibodies, contacting *E. coli* and *H. influenza* with Bio-amp and determining the biological activity thereof. This rejection is respectfully traversed.

Independent claims 1 and 3 are drawn to a method for introducing a compound into a Gram negative bacterial cell and independent claim 5 recites contacting a Gram negative bacterial cell with biotinylated compound to cause uptake of the biotinylated compound by the cell. In other words, the compound of claims 1, 3 and 5 is internalized by the cell. The biotinylated compounds described in Dragis et al., on the other hand, are not internalized by the cell. For example, at page 973, left-hand column, Dargis et al. teach that "beta-lactam drugs must bind to specific targets located in the cytoplasmic membrane of bacteria." In addition, Dargis et al. tested for expression of the beta-lactams specifically in the bacterial membranes. The method for isolating the bacterial membrane is disclosed on page 973, right-hand column. Therefore, the compound taught by Dargis et al. merely binds to the cell membrane and does not get introduced *into a cell* (claims 1 and 3, and those dependent therefrom) or cause *uptake* of the biotinylated compound by the cell as recited in claim 5.

For at least the reasons given above, Applicants submit that Dargis et al. fail to teach each and every element of the rejected claims. Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-8, 22-26, 29 and 32-35 as being anticipated by Dargis et al.

Rejection under 35 U.S.C. §103(a)

The Examiner has rejected claims 1-8, 21-26, 29 and 32-35 under 35 U.S.C. 103(a) as being unpatentable over Low et al. This rejection is respectfully traversed.

The Examiner asserts that it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to substitute biotin-linked antimicrobial peptides, peptide antibiotics, or antibiotics of Low et al. for the pUC8 nucleic acid in the method of delivery to *E. coli* of Low et al. in the absence of $CaCl_2$ because Low et al. teach that biotin mediated transport is effective for delivery of any of the desired exogenous compounds and proteins and peptides in particular in the absence of $CaCl_2$.

Applicants respectfully disagree. First, as noted above and contrary to the Examiner's interpretation of Low et al., the *E. coli* example in Low et al., Example 12, does in fact teach the use of membrane permeablizing agent, namely, $CaCl_2$. This is significant, because although claims 1 and 3 are drawn to methods performed using a Gram negative bacterial cell, they recite the *absence* of a permeablizing agent.

Moreover, there are many important differences between and among the cellular structures of plant, mammalian, and bacterial/prokaryotic cells, including the cell wall and/or cell membrane. The skilled artisan would certainly not expect these varied cell types to function similarly with respect to uptake of foreign matter. More specifically, the skilled artisan would appreciate that even if mammalian and plant cells may indeed be capable of taking up various compounds in the absence of a membrane permeabilizing agent, a Gram negative bacterial cell may not function in the same manner. The outer membrane of Gram negative bacteria is recognized in the art as an effective permeability barrier. It contains a highly ordered quasicrystalline structure with very low fluidity (Vaara et al., "Outer Membrane Permeability Barrier in *Escherichia coli* Mutants That Are Defective in the Late Acyltransferases of Lipid A Biosynthesis" Antimicrob Agents Chemother. 1999 June; 43(6): 1459–1462). The teachings of Low et al. are conventional with respect to Gram negative bacterial cells in that they assume the use of a premeabilizing agent when the target cell is *E. coli*. Low et al. fail to even suggest the introduction of any compound into bacterial cells in the absence of a membrane-permeabilizing agent. The cited art would not lead one of skill in the art to reasonably expect that peptides could

be successfully introduced into Gram negative bacterial cells *in the absence* of a membrane permeabilizing agent.

With respect to claim 5, the Examiner asserts that it would have been obvious to further test for the antimicrobial activity of the delivered compounds as a means of assessing the effectiveness of delivery of antimicrobial agents.

Claim 5 recites a method for identifying a compound having antimicrobial activity comprising contacting a Gram negative bacterial cell with biotinylated compound to cause uptake of the biotinylated compound by the cell and determining whether the biotinylated compound has an antimicrobial effect on the cell. Stated another way, claim 5 is directed to utilizing the method of biotin-facilitated uptake to introduce candidate compounds into the cell and to screen those candidate compounds for antimicrobial effects. Applicants respectfully submit that Low et al. do not teach, or even suggest, a method for identifying a compound having antimicrobial activity as recited in claim 5; as noted above the DNA they introduced into *E. coli* confers antibiotic resistance, not activity.

For at least the reasons set forth above, it is respectfully submitted that claims 1-8, 21-26, 29 and 32-35 are not obvious in view of Low et al. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-8, 21-26, 29 and 32-35 under 35 U.S.C. 103(a) as being unpatentable over Low et al.

The Examiner rejected claims 27 and 28 as being unpatentable over Low et al., as applied to claims 1-8, 21-26, 29 and 32-35, in view of Kim (U.S. Patent 6,322,788). Kim is cited by the Examiner as teaching anti-bacterial antibodies conjugated to antibiotics, and that the advantage of the antibiotic conjugate is that the antibody targeting moiety provides for concentrated localized delivery of the antibiotic. This rejection is respectfully traversed.

Claims 27 and 28 depend indirectly from claims 1, 3 and 5. For reasons set forth above, Applicant submit that claims 1, 3 and 5 are novel and nonobvious over the cited art. It is

therefore submitted that claims 27 and 28, which depend therefrom, are therefore also nonobvious over the cited art.

Furthermore, it should be noted that the biotinylated compounds of the present invention are internalized by the Gram negative bacterial cell. In contrast, the antibiotic/antibody conjugate taught in Kim binds to the cell surface of an animal or human cell and there is no evidence that the conjugate is internalized. In fact, there are no working examples at all in Kim. Additionally, claims 27 and 28 recite a biotinylated compound that further comprises a targeting moiety that specifically targets a Gram negative bacterial cell. In contrast, the antibiotic/antibody conjugate taught in Kim is stated as binding to Fc-binding proteins from Gram *positive* bacteria (i.e., *Staphylococcus aureus* and *Streptococci* sp) or, alternatively, directly to the Gram positive bacteria, not Gram *negative* bacteria as recited in claims 27 and 28.

For at least the reasons set forth above, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 27 and 28 under 35 U.S.C. 103(a) as being unpatentable over Low et al., as applied to claims 1-8, 21-26, 29 and 32-35, in view of Kim (U.S. Patent 6,322,788).

Summary

It is respectfully submitted that the pending claims 1-8, 21-29, 32-35, and 43 are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Representatives at the telephone number listed below if it is believed that prosecution of this application may be assisted thereby.

CERTIFICATE UNDER 37 C.F.R. 1.8:

The undersigned hereby certifies that the paper(s), as described hereinabove, are being transmitted via the U.S. Patent and Trademark Office electronic filing system in accordance with 37 CFR §1.6(a)(4) to the Patent and Trademark Office addressed to the Commissioner for Patents, Mail Stop Amendment, P.O. Box 1450, Alexandria, VA 22313-1450,
on this 17 day of November, 2008.

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